A. FRONT COVER/TITLE PAGE

TITLE OF RESEARCH PROJECT:

ASSESSING THE EFFECTS OF SOIL HUMIC AND FULVIC ACIDS ON GERMINATION AND EARLY GROWTH OF NATIVE AND INTRODUCED GRASS VARIETIES

NAME OF PRINCIPAL INVESTIGATOR: SENESI NICOLA-PROFESSOR

NAME OF CONTRACTOR: UNIVERSITA' DI BARI

CONTRACT NUMBER: N62558-05-P-0179

PURCHASE REQUEST NUMBER: W90C2K9889EN01

2nd INTERIM REPORT

REPORT PERIOD: SEPTEMBER 22, 2005 – MARCH 22, 2006

END OF FRONT COVER/TITLE PAGE

REPORT DOCUMENTATION PAGE	Form Approved OMB No. 0704-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	6 December 2005	2ND Interim Report, September 22, 2005 – March 22, 2006

4. TITLE AND SUBTITLE

ASSESSING THE EFFECTS OF SOIL HUMIC AND FULIC ACIDS ON GERMINATION AND EARLY GROWTH OF NATIVE AND INTRODUCED GRASS VARITIES, 2ND INTERIM REPORT

5. FUNDING NUMBERS

Contract Number N62558-05-P-0179

6. AUTHOR(S)

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8. Performing Organization Report Number

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES

Engineer Research and Development Center , Cold Regions Research & Engineering Laboratory, 72 Lyme Road, Hanover, New Hampshire, 03755-1290, USA.

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT	12b. DISTRIBUTION CODE
Approved for public release; distribution is unlimited.	A

ABSTRACT (Maximum 200 words)

SCIENTIFIC WORK DONE DURING THE REPORTING PERIOD

Chemical and Spectroscopic Characterization of Original and Greenhouse Control Soil Humic Acids

Experimental

Five humic acid (HA) samples isolated from the two **original (collected in the field)** Wyoming soils in duplicate, **Guernsey North (GN4+5 and GN6) and Guernsey South (GS4+5 and GS6)**, and one Utah soil, **Dugway (D11+12+13+14)**, and **seven HA** samples isolated from the same soils but collected in duplicate or triplicate from control (no plants grown) **greenhouse pots (GN-A and GN-B, GS-A and GS-B, D-A, D-B and D-C)**, object of this research, were obtained from the USDA-St.Paul group. A solution 0.5 M NaOH was used to extract these HAs from soils.

All HA samples were characterized for moisture and ash contents, elemental (C, H, N, S, O) and acidic functional group (total acidity, COOH, phenolic OH) composition, and by Fourier transform infrared (FT IR) spectroscopy, and fluorescence spectroscopy in the emission, excitation and synchronous scan modes.

RESEARCH PLANS FOR THE REMAINDER OF THE CONTRACT PERIOD

For the remainder of the contract period (14 months) research plans are the following:

(a) Chemical and spectroscopic characterization of the HAs isolated in replicates from greenhouse pot soils where the two grass/four varieties were grown.

(b) Germination and early growth experiments of the four grass varieties in combinations by two in the presence of the three soil HAs at two concentrations.

(c) Possible follow-up experiments with HA concentrations optimal to promote the growth of the grass varieties of interest.

(d) Correlation of the germination and seedling growth data with the chemical and spectroscopic parameters of the HAs examined, in order to possibly find out which HA parameters may influence germination and growth of the four grass varieties examined.

14. SUBJECT TERMS	15. NUMBER OF PAGES
Navy, Italy, Soil Humic acids, Soils, Greenhouse, Acidic functional group, Wheatgrass, Germination	
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19, SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED	UL

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. 239-18
298-102

B. BODY OF THE REPORT

(1) SCIENTIFIC WORK DONE DURING THE REPORTING PERIOD

1. Chemical and Spectroscopic Characterization of Original and Greenhouse Control Soil Humic Acids

1.1. Experimental

Five humic acid (HA) samples isolated from the two **original (collected in the field)** Wyoming soils in duplicate, **Guernsey North (GN4+5 and GN6) and Guernsey South (GS4+5 and GS6)**, and one Utah soil, **Dugway (D11+12+13+14)**, and **seven HA** samples isolated from the same soils but collected in duplicate or triplicate from control (no plants grown) **greenhouse pots (GN-A and GN-B, GS-A and GS-B, D-A, D-B and D-C)**, object of this research, were obtained from the USDA-St.Paul group. A solution 0.5 M NaOH was used to extract these HAs from soils.
All HA samples were characterized for moisture and ash contents, elemental (C, H, N, S, O) and acidic functional group (total acidity, COOH, phenolic OH) composition, and by Fourier transform infrared (FT IR) spectroscopy, and fluorescence spectroscopy in the emission, excitation and synchronous scan modes.

1.2. Results and Discussion

The **elemental composition** (C, H, N, S, O), calculated **atomic ratios** and **acidic functional group** contents, on a moisture- and ash-free basis, of the five original soil HAs elencated above are shown, respectively, in **Tables 1, 2, and 3**, whereas **Tables 4, 5, and 6** show the same parameters for the seven greenhouse control soil HAs. The **FT IR** spectra of the five original soil HAs and the seven greenhouse soil HAs are shown, respectively, in **Figs. 1 and 5**, whereas the **fluorescence** emission, excitation and synchronous scan spectra of these samples are shown in **Figs. 2, 3, 4, and 6, 7, 8,** respectively.
The elemental and acidic functional group composition of all HAs studied are typical of soil HAs. With the exception of moisture, ash, phenolic OH and total acidity contents, no significant differences are shown between duplicates of each soil HA analysed. In general, the D-HAs feature C, N, and H contents higher and S and O contents and C/N ratio lower than those of GN-HAs and GS-HAs. With some exception, e.g., phenolic OH and total acidity contents and C/N ratio, the element and functional group content of GN-HAs are similar to those of GS-HAs. With some exceptions, no relevant differences in the elemental and functional group composition is also shown between the corresponding HAs of the two series of five and seven HAs.
The FT IR spectra of all HAs are generally qualitatively similar one to another, and typical of soil HAs, featuring absorption bands at similar wavenumber values but often showing slightly variable relative intensity. The FT IR spectra of the HAs from soils GS 4+5, GN B, GS A, GS B, and D B, feature, however, a sharp and intense band at about 1100 cm^{-1}, possibly ascribed to Si-O stretching of clay mineral impurities coextracted with the HA.
The emission, excitation and synchronous scan fluorescence spectra of all HAs examined are similar one to another, and typical of well humified soil HAs. The emission spectra feature a unique broad maximum at a wavelength that is longer for GS HAs (540-545 nm) than for the other HAs (525-535 nm) The synchronous scan spectra also show a unique broad peak, often with a shoulder extending to longer wavelengths, which is centered at shorter wavelengths (488-489 nm) for GN HAs, at intermediate wavelengths (501-508 nm) for D HAs, and at longer wavelengths (514-517 nm) for GS HAs. The excitation spectraof the HAs studied are similar one to another,

featuring an intense peak at a wavelength ranging from 447 to 450 nm. These results would suggest a higher humification degree for GS HAs with respect to the other HAs studied.

2. Germination and Early Growth of the Vavilov and SERDP-Select varieties of Siberian Wheatgrass

2.1. Experimental

2.1.1. Plant species and varieties

In order to have comparable results, in the second phase of this research, the germination and early growth of the other **two varieties** of interest, the **cv. Vavilov** and the **germplasm line SERDP-select** of the **introduced plant species siberian wheatgrass**, were studied using the same three HA samples (**GN-HA**, **GS-HA** and **D-HA)** at the same concentrations of 10 and 100 mg/L, in the same conditions and using the same experimental protocol used in the first set of experiments performed with the cv. Pryor and the germplasm line SERD-select of the native plant slender wheatgrass, and described in the 1st Report of this research Project.

2.1.2. Germination experiment

The seeds were preliminary surface-sterilized by dipping them for 15 min in sodium hypoclorite 0.2 %, and then washing several times with distilled water. Twenty (20) seeds for each of the five (5) replicates were placed in Petri dishes on filter paper, and added with suspensions of each HA at each concentration in distilled water, or with distilled water only (control). The Petri dishes were kept in the dark for 6 days in a thermostated chamber at a temperature of 20 °C. After this time period, germinated seeds were removed and counted, and the lengths of the primary root and shoot were measured.

2.1.3. Early growth experiment

After the end of the germination experiment and collection of germination data, the germinated seeds (seedlings) of the two siberian wheatgrass varieties were inserted into holes of aluminum lids placed on the top of glass pots (5 seedlings per pot). The pots were filled with the Nitch nutrient solution, in the absence (control) or presence of each HA at concentrations of 10 and 100 mg/L. The pH of the nutrient solution was preliminarly adjusted to 6.5 with a solution of NH_4OH. Blanks (without seedlings) were also prepared for each treatment in order to measure the pH change during the growth period in the absence of plants. The pH of all treatment media ranged between 6.5 (control) and 5.9 (GS-HA at 100 mg/L). The pots were then placed in a Phytotron growth chamber, and seedlings were allowed to grow for a period of 21 days in the following conditions: (a) photoperiod of 12-h; (b) temperature of 20 °C and humidity of 74% during the illumination period; and (c) temperature of 17 °C and humidity of 70% during the dark period. At the end of the experiment, the pH of the growth solutions and blanks, and the length and fresh and dry weights (60 °C for 48 h) of roots and shoots were measured. All experiments were conducted in five replicates.

2.1.4. Statistical analyses

All germination and growth data were analyzed statistically by one-way analysis of variance (ANOVA) and the means of the treatments were separated by the least significant difference (LSD) test.

2.2. Results and Discussion

2.2.1. Germination data

Statistical treatment of data by one-way analysis of variance shows, with respect to the corresponding controls, and as a function of either the HA type or HA concentration (Table 7), the existence of: (a) a non significant difference in the germination percentages (%) of the two siberian wheatgrass varieties; and (b) a highly significant or significant difference in the length of primary root and shoot of germinated seeds of both varieties.
Numerical data in Table 8 and Fig. 9 suggest, however,that the three HAs at both concentrations exert a general slight positive effect in promoting germination of both siberian wheatgrass varieties. Further, numerical data in Table 9 and Fig. 10 suggest that, with respect to the control: (a) both primary root length, especially, and primary shoot length of the germplasm line SERDP-select are significantly or highly significantly increased by any HA at both concentrations; and (b) at both concentrations D-HA does not affect and GS-HA promotes the primary root and shoot lengths of the cv. Vavilov, whereas GN-HA has a positive effect only at the highest concentration on shoot primary length.
In conclusion, although the germination and primary shoot and root growth of the two varieties of siberian wheatgrass appear to be affected in different ways and at different extent by the HA origin and concentration, the germplasm line SERDP-select is affected positively by any HA at any concentration used, whereas the cv. Vavilov is only in some cases positively affected by the presence of HA.

2.2.2. pH of the growth medium

Statistical treatment of data by one-way analysis of variance shows that, with respect to the corresponding controls, the pH of the medium experiences no significant variation during the 21-day growth period of both the cv. Vavilov and the germplasm line SERDP-select (Table 7). Also, no pH variation is measured for the blanks (no plants present) during the same period of time.
Data in Fig.11 show, however, that in the presence of the germplasm line SERDP-select a slight acidification of the growth medium occurs with any HA at any concentration.

2.2.3. Early growth data

Statistical treatment of all experimental data by one-way analysis of variance (Table 7) shows that differences exist between the treatments and the control for any parameter measured, which are generally highly significant for the germplasm line SERDP-select and significant for the cv. Vavilov, with the exception of shoot length of the latter.

Numerical data in Table 10 (expressed in cm) and Fig. 12 (expressed in %) indicate that: (a) the root length of the cv. Vavilov is depressed highly significantly by the presence of any HA at any concentration, with the exception of D-HA at 10 mg/L, whereas the shoot length is generally unaffected or slightly depressed; and (b) any HA at low concentration (10 mg/L) significantly increases the root length, with the exception of GN-HA, and highly significantly stimulates the shoot length of the germplasm line SERDP-select, whereas at high concentration only a slight non significant positive effect is shown by any HA on both root and shoot lengths.

Numerical data in Table 11 (expressed in mg) and Fig. 13 (expressed in %) show that : (a) the fresh weights of the shoots and roots of the cv. Vavilov are generally reduced, and in some cases significantly, by the presence of HA, and vary on dependence of the HA source and concentration;

(b) on the contrary, the root fresh weight of the germplasm line SERDP-select generally increases highly significantly in the presence of any HA at any concentration, and the shoot fresh weigth is increased by any HA highly significantly at low concentration, and slightly at high concentration.

Numerical data in Table 12 (expressed in mg) and Fig. 14 (expressed in %) confirm those presented above for root and shoot fresh weights. In particular, any HA at any concentration generally produces no effect or a decrease, in some cases significant, of the root and shoot dry weights of the cv. Vavilov, and an increase, in some cases significant, of those of the germplasm line SERDP-select.

2.3. Conclusions

In conclusion, not only the germination, but also the early growth, including root and shoot lengths and fresh and dry weights, of the germplasm line SERDP-select of siberian wheatgrass is affected positively and at various extents by the presence of HAs, whereas the response of the cv. Vavilov in the same growth conditions is generally negative or indifferent.

(2) RESEARCH PLANS FOR THE REMAINDER OF THE CONTRACT PERIOD

For the remainder of the contract period (14 months) research plans are the following:

(a) Chemical and spectroscopic characterization of the HAs isolated in replicates from greenhouse pot soils where the two grass/four varieties were grown.
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(d) Correlation of the germination and seedling growth data with the chemical and spectroscopic parameters of the HAs examined, in order to possibly find out which HA parameters may influence germination and growth of the four grass varieties examined.

(3) SIGNIFICANT ADMINISTRATIVE ACTIONS DURING THE PERIOD REPORTED: NONE.

(4) ANY OTHER INFORMATION : NONE.

(5) ANNEX

(A) AMOUNT OF UNUSED FUNDS REMAINING ON THE CONTRACT AT THE END OF THE PERIOD COVERED BY THE REPORT: US$ 20,000.
(B) IMPORTANT PROPERTIES ACQUIRED WITH CONTRACT DURING THIS PERIOD: NONE.
(C) METHOD OF REPRODUCTION: E-MAIL ATTACHMENTS, PHOTOCOPYING.

Table 1. Elemental composition (on moisture and ash-free basis) of humic acids isolated from the five original field soils with 0.5 M NaOH.

Origin of humic acids	**Moisture**	**Ash**	**C**	**N**	**H**	**S**	**O (*)**
	%	**%**	**%**	**%**	**%**	**%**	**%**
GN 4+5	5.4	1.1	56.6 ± 0.3	4.9 ± 0.1	5.4 ± 0.1	0.4 ±0.1	32.7
GN 6	3.4	0.4	56.1 ±0.3	4.9 ±0.1	5.4 ± 0.3	0.4 ±0.1	33.2
GS 4+5	5.2	7.2	57.4 ± 0.3	4.7 ± 0.1	5.4 ± 0.1	0.4 ±0.1	32.1
GS 6	9.3	0.7	58.3 ±0.1	4.8 ±0.1	5.0 ± 0.1	0.3 ±0.1	31.6
D 11+12+13+14	7.2	6.6	58.2 ± 0.5	5.4 ± 0.1	5.8 ± 0.1	0.3 ±0.1	30.3

(*) Oxygen was obtained by difference to 100

Table 2. Atomic ratios (calculated on the basis of data in Table 1) of humic acids isolated from the five original field soils with 0.5 M NaOH.

Origin of humic acids	**C/N**	**C/H**	**O/C**
GN 4+5	13.5	0.9	0.4
GN 6	13.5	0.9	0.4
GS 4+5	14.2	0.9	0.4
GS 6	14.3	1.0	0.4
D 11+12+13+14	12.6	0.8	0.4

Table 3. Acidic functional group content (on moisture and ash-free basis) of humic acids isolated from the five original field soils with 0.5 M NaOH.

Origin of humic acids	**COOH**	**Phen. OH**	**Tot. Ac.**
	meq/g	**meq/g**	**meq/g**
GN 4+5	3.5	3.8	7.3
GN 6	3.3	1.8	5.1
GS 4+5	3.9	3.4	7.3
GS 6	3.7	2.0	5.7
D 11+12+13+14	3.9	2.2	6.1

Table 4. Elemental composition (on moisture and ash-free basis) of humic acids isolated from the seven control greenhouse soils with 0.5 M NaOH.

Origin of humic acids	**Moisture**	**Ash**	**C**	**N**	**H**	**S**	**O (*)**
	%	**%**	**%**	**%**	**%**	**%**	**%**
GN A	5.4	3.3	55.2 ± 0.7	4.9 ± 0.1	5.2 ± 0.1	1.7 ± 0.1	33.0
GN B	3.5	8.0	55.0 ± 0.6	4.8 ± 0.1	5.1 ± 0.1	0.5 ± 0.1	34.6
GS A	5.0	9.2	55.5 ± 1.3	4.7 ± 0.1	4.9 ± 0.2	0.5 ± 0.1	34.48
GS B	4.2	8.1	56.5 ± 0.7	4.9 ± 0.06	5.1 ± 0.1	0.5 ± 0.1	33.08
D A	4.5	1.8	56.9 ±1.7	5.2 ±0.1	5.4 ± 0.1	0.3 ±0.1	32.2
D B	5.6	9.5	56.2 ±0.1	5.1 ±0.1	5.5 ±0.1	0.3 ±0.1	32.9
D C	7.4	1.5	58.4 ±0.7	5.4 ±0.1	5.6 ±0.1	0.3 ±0.1	30.3

(*) Oxygen was obtained by difference to 100

Table 5. Atomic ratios (calculated on the basis of data in Table 1) of humic acids isolated from the seven control greenhouse soils with 0.5 M NaOH.

Origin of humic acids	**C/N**	**C/H**	**O/C**
GN A	13.1	0.9	0.5
GN B	13.6	0.9	0.5
GS A	13.9	0.9	0.5
GS B	13.4	0.9	0.5
D A	12.7	0.9	0.4
D B	12.7	0.9	0.4
D C	12.5	0.9	0.4

Table 6. Acidic functional group content (on moisture and ash-free basis) of humic acids isolated from the seven control greenhouse soils with 0.5 M NaOH.

Origin of humic acids	COOH	Phen. OH	Tot. Ac.
	meq/g	meq/g	meq/g
GN A	3.2	1.7	4.90
GN B	3.5	1.7	5.2
GS A	3.9	0.2	4.1
GS B	3.6	0.6	4.2
D A	3.9	1.7	5.6
D B	4.2	2.0	6.2
D C	3.8	1.2	5.0

Table 7. Significance level (F value) resulting from one-way Analysis of Variance (ANOVA) of all data obtained for each parameter measured distinctly for plant species.

Parameter	Siberian Wheatgrass Vavilov	Siberian Wheatgrass SERDP-select
Germination	0.80 ^{ns}	1.15 ^{ns}
Primary root length	3.69 **^{s}	6.20 ***
Primary shoot length	9.04 ***	2.77 *
pH	1.17 ^{ns}	2.29 ^{ns}
Root length	5.38 ***	2.72 *
Shoot length	1.37 ^{ns}	6.09 ***
Root fresh weight	3.31 *	9.42 ***
Shoot fresh weight	3.16 *	5.94 ***
Root dry weight	3.00 *	4.46 **
Shoot dry weight	2.58 *	5.55 ***

*** 0.001; ** 0.01 P; * 0.05 P; ns: nonsignificant

Table 8. Effect of HAs at different concentrations on seed germination (percentage of germinated seeds ± standard error for five replicates) measured immediately before transplanting.

Parameter	Siberian Wheatgrass Vavilov	Siberian Wheatgrass SERDP-select
Control (H_2O)	58 ± 4.60	65 ± 4.24
D HA		
10 mg/L	70 ± 3.74	72 ± 2.28
100 mg/L	60 ± 5.10	79 ± 2.61
GS HA		
10 mg/L	67 ± 1.79	67 ± 5.93
100 mg/L	67 ± 3.90	74 ± 5.18
GN HA		
10 mg/L	68 ± 4.82	67 ± 3.03
100 mg/L	63 ± 5.40	74 ± 4.56

Table 9. Effect of HAs at different concentrations on the length (cm ± standard error for five replicates) of primary root and primary shoot of germinated seeds.

Parameter	Siberian Wheatgrass Vavilov		Siberian Wheatgrass SERDP-select	
	Root	Shoot	Root	Shoot
Control (H_2O)	2.7 ± 0.1	2.2 ± 0.1	1.9 ± 0.1	2.2 ± 0.1
D HA				
10 mg/L	2.8 ± 0.1	2.0 ± 0.1	2.2 ± 0.1	2.6 ± 0.1
100 mg/L	2.7 ± 0.2	1.9 ± 0.1	2.9 ± 0.1	2.9 ± 0.1
GS HA				
10 mg/L	3.0 ± 0.1	2.7 ± 0.1	2.4 ± 0.1	2.7 ± 0.1
100 mg/L	3.6 ± 0.1	3.1 ± 0.1	2.5 ± 0.1	2.7 ± 0.1
GN HA				
10 mg/L	2.8 ± 0.2	2.3 ± 0.2	2.4 ± 0.1	2.8 ± 0.1
100 mg/L	3.0 ± 0.1	2.8 ± 0.1	2.7 ± 0.1	2.6 ± 0.2

Table 10. Effect of HAs at different concentrations on the length (cm ± standard error for five replicates) of shoots and roots measured after 21-day growth.

Treatment	Siberian Wheatgrass Vavilov		Siberian Wheatgrass SERDP-select	
	Root	Shoot	Root	Shoot
Control (H_2O)	7.5 ± 0.1	9.4 ± 0.1	9.4 ± 0.8	2.7 ± 0.1
D HA				
10 mg/L	7.2 ± 0.7	9.1 ± 0.3	12.9 ± 0.7	4.2 ± 0.2
100 mg/L	5.6 ± 0.2	7.6 ± 0.3	11.2 ± 0.5	2.6 ± 0.1
GS HA				
10 mg/L	5.1 ± 0.2	7.9 ± 0.4	12.3 ± 0.6	3.8 ± 0.3
100 mg/L	4.9 ± 0.4	9.1 ± 0.8	9.8 ± 0.8	3.3 ± 0.1
GN HA				
10 mg/L	5.7 ± 0.4	9.2 ± 0.7	10.8 ± 0.8	4.3 ± 0.5
100 mg/L	5.6 ± 0.5	8.4 ± 0.8	10.0 ± 0.7	3.0 ± 0.1

Table 11. Effect of HAs at different concentrations on the fresh weight (mg ± standard error for five replicates) of shoots and roots measured after 21-day growth.

Treatment	Siberian Wheatgrass Vavilov		Siberian Wheatgrass SERDP-select	
	Root	Shoot	Root	Shoot
Control (H_2O)	5.3 ± 0.1	10.7 ± 0.6	11.6 ± 1.9	35.4 ± 4.1
D HA				
10 mg/L	5.5 ± 0.6	10.1 ± 0.7	55.9 ± 6.9	98.3 ± 12.9
100 mg/L	5.8 ± 0.5	7.6 ± 0.7	26.9 ± 3.4	30.3 ± 5.4
GS HA				
10 mg/L	3.4 ± 0.1	6.5 ± 0.8	37.0 ± 2.9	51.1 ± 9.6
100 mg/L	3.8 ± 0.4	8.5 ± 1.2	53.4 ± 6.4	98.3 ± 15.8
GN HA				
10 mg/L	7.1 ± 0.6	8.7 ± 0.6	49.7 ± 2.2	90.7 ± 10.6
100 mg/L	4.2 ± 0.4	7.2 ± 0.3	49.1 ± 6.5	64.5 ± 11.7

Table 12. Effect of HAs at different concentrations on the dry weight (mg ± standard error for five replicates) of shoots and roots measured after 21-day growth.

Treatment	Siberian Wheatgrass Vavilov		Siberian Wheatgrass SERDP-select	
	Root	Shoot	Root	Shoot
Control (H_2O)	1.7 ± 0.1	1.5 ± 0.1	2.0 ± 0.3	8.0 ± 1.0
D HA				
10 mg/L	1.6 ± 0.1	1.5 ± 0.1	3.8 ± 0.4	15.8 ± 1.8
100 mg/L	1.6 ± 0.1	1.1 ± 0.1	2.0 ± 0.2	6.0 ± 0.6
GS HA				
10 mg/L	1.1 ± 0.1	1.1 ± 0.1	2.2 ± 0.2	8.4 ± 0.9
100 mg/L	1.3 ± 0.1	1.2 ± 0.2	3.0 ± 0.3	14.3 ± 2.2
GN HA				
10 mg/L	1.4 ± 0.2	1.3 ± 0.2	3.0 ± 0.3	14.3 ± 1.3
100 mg/L	1.5 ± 0.1	1.2 ± 0.1	2.1 ± 0.1	9.3 ± 1.7

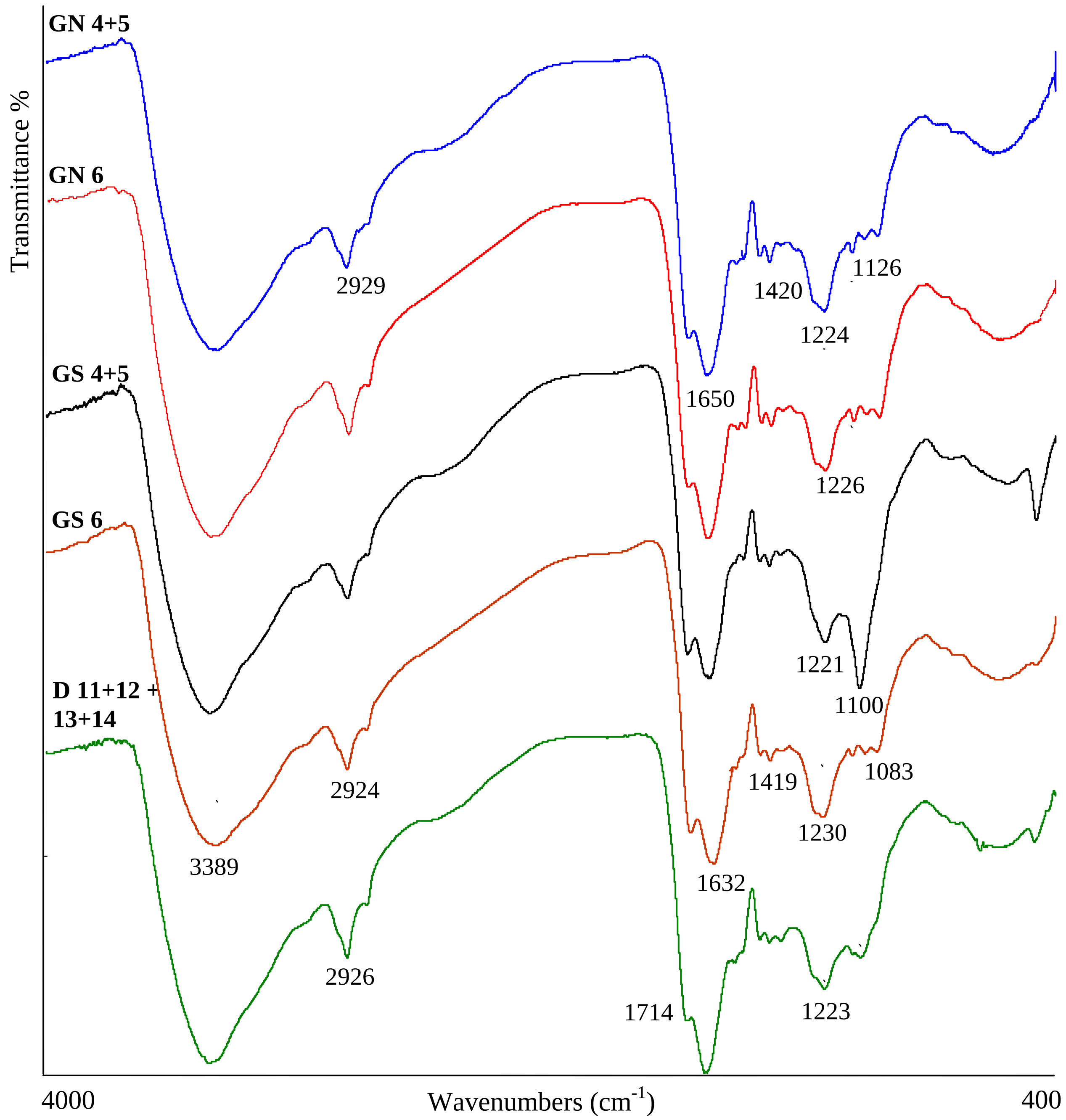


Figure 1. FT IR spectra of the humic acids isolated from the five original field soils.

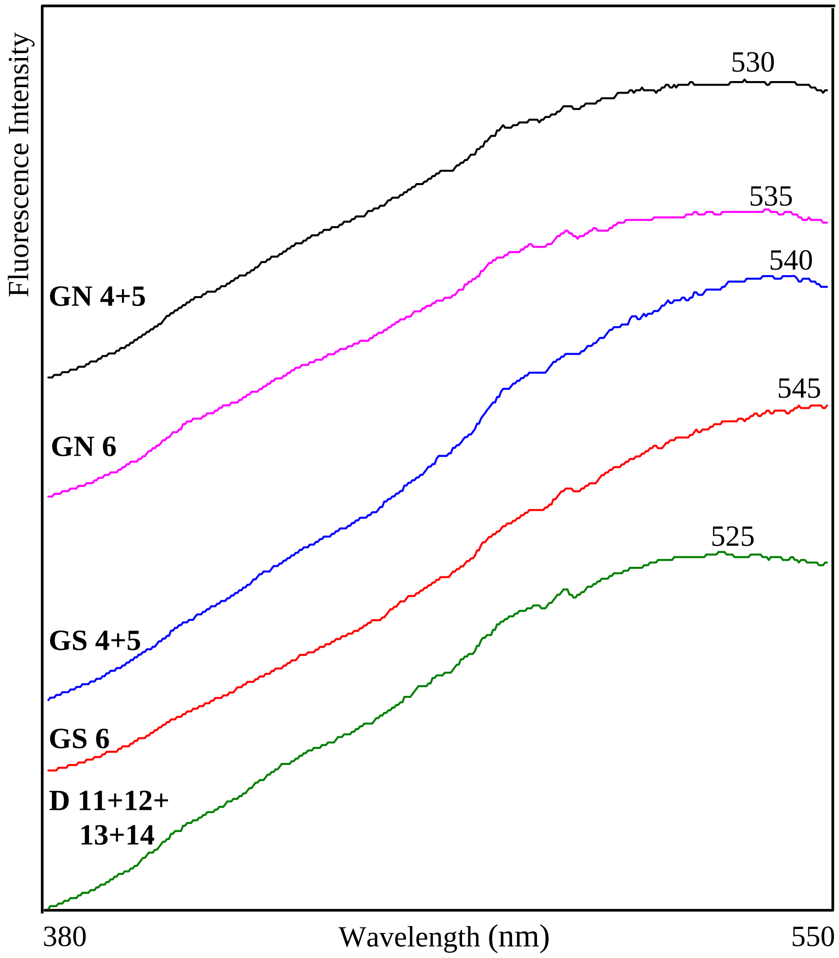


Figure 2. Fluorescence emission spectra of the humic acids isolated from the five original field soils.

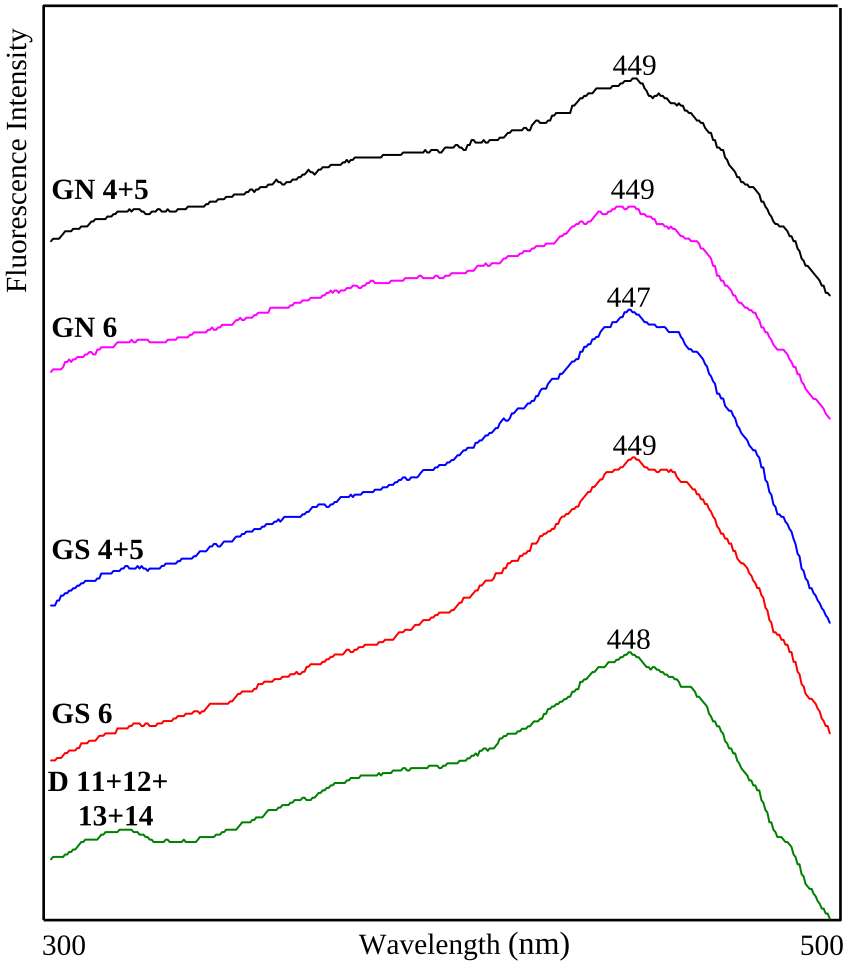


Figure 3. Fluorescence excitation spectra of the humic acids isolated from the five original field soils.

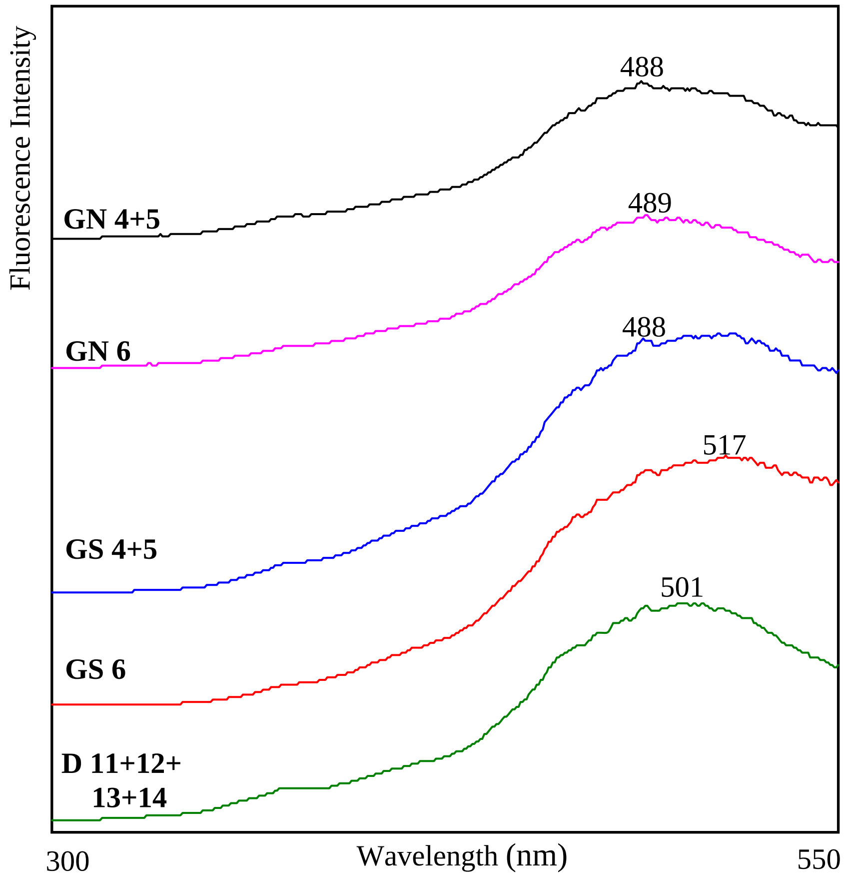


Figure 4. Fluorescence synchronous scan spectra of the humic acids isolated from the five original field soils.

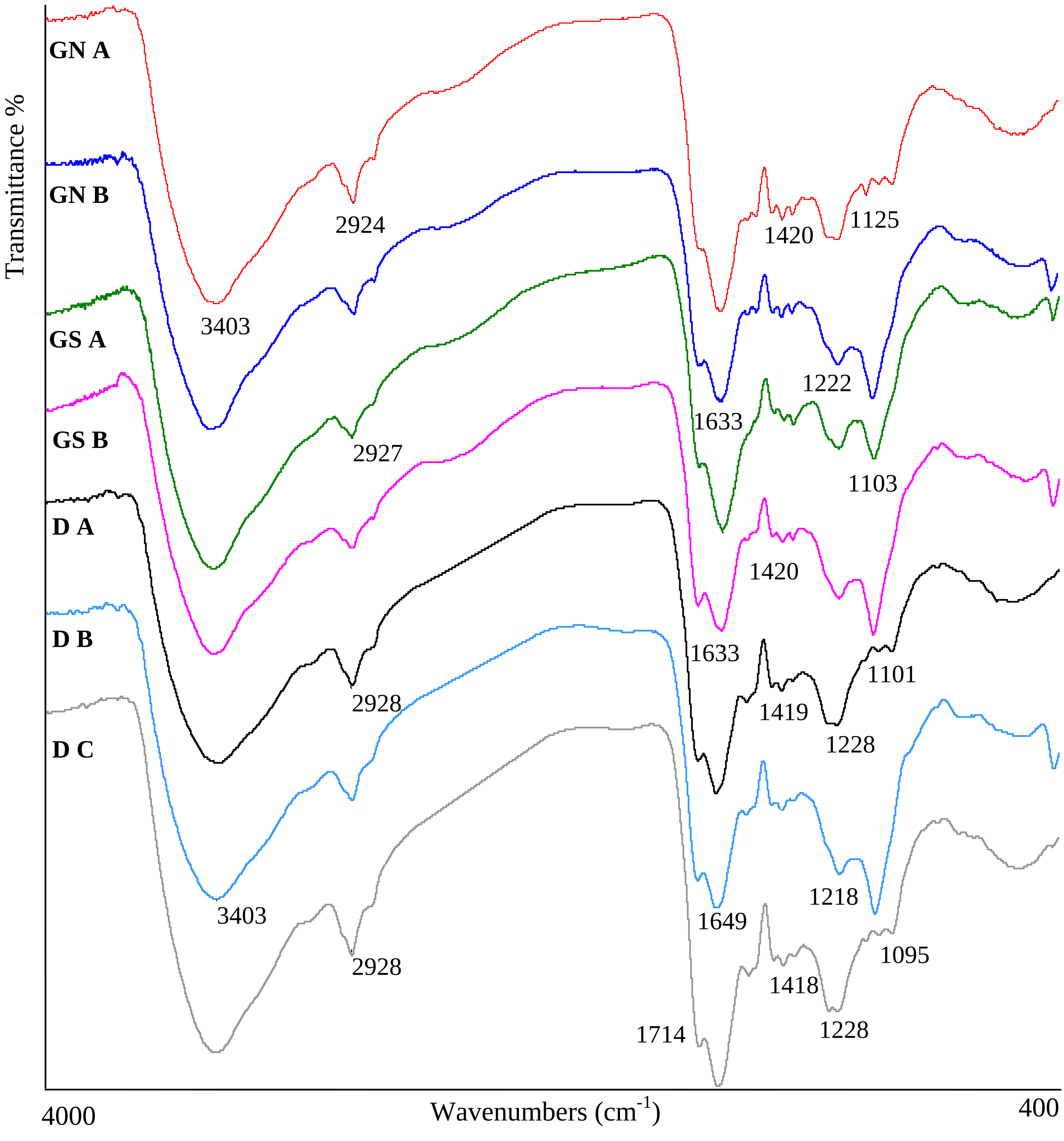


Figure 5. FT IR spectra of the humic acids isolated from the seven control greenhouse soils.

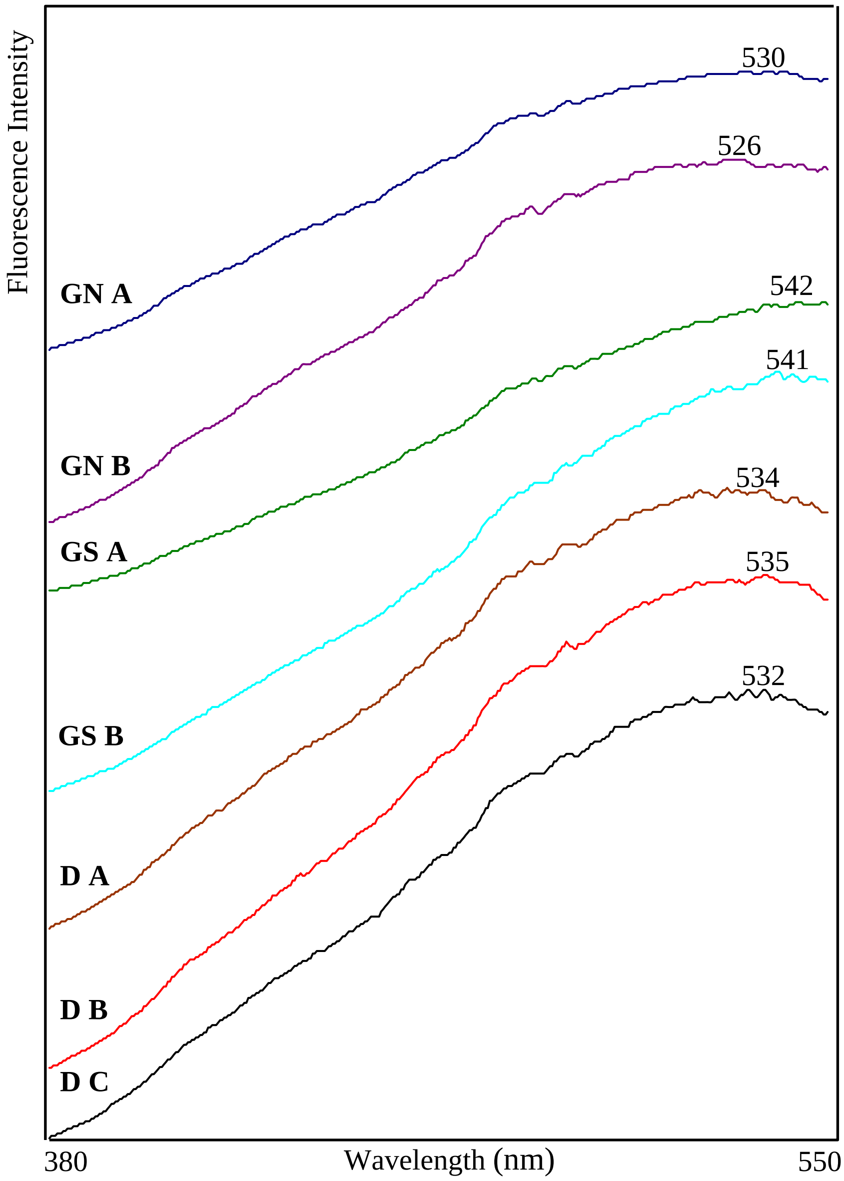


Figure 6. Fluorescence emission spectra of the humic acids isolated from the seven control greenhouse soils.

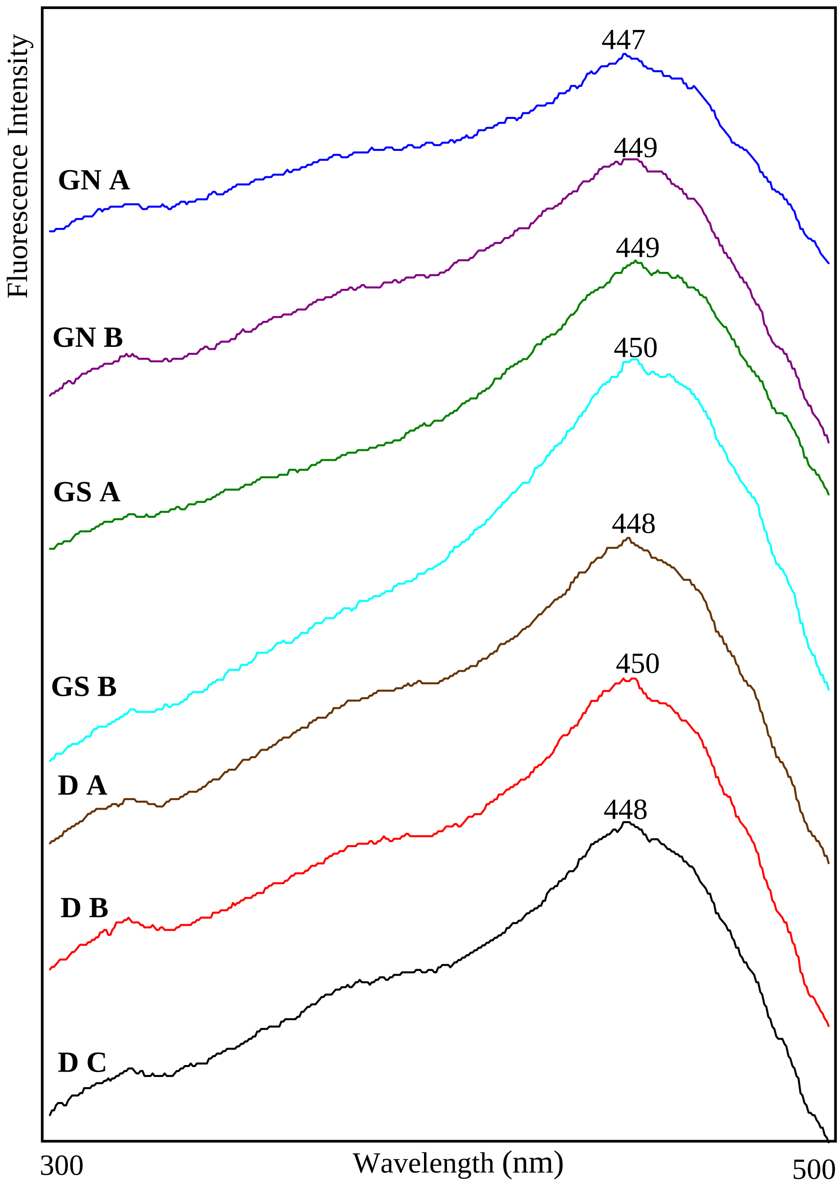


Figure 7. Fluorescence excitation spectra of the humic acids isolated from the seven control greenhouse soils.

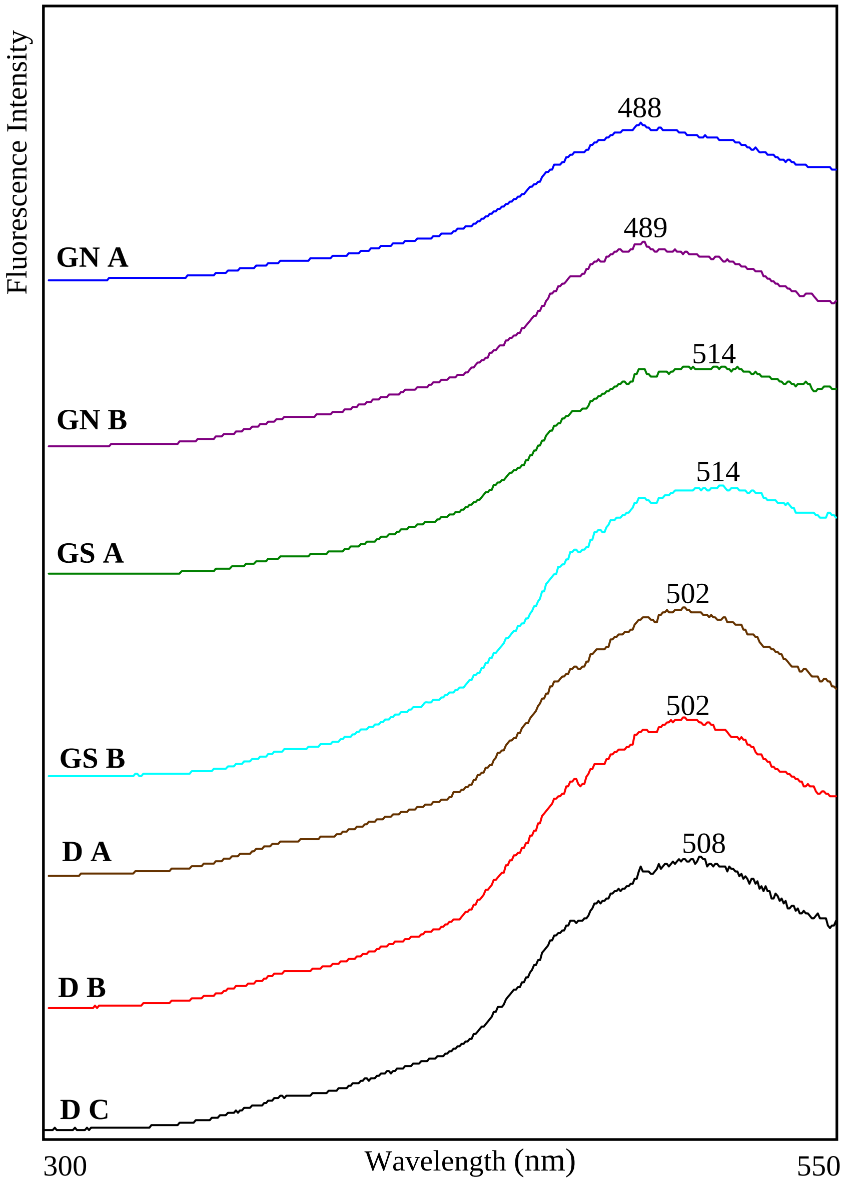
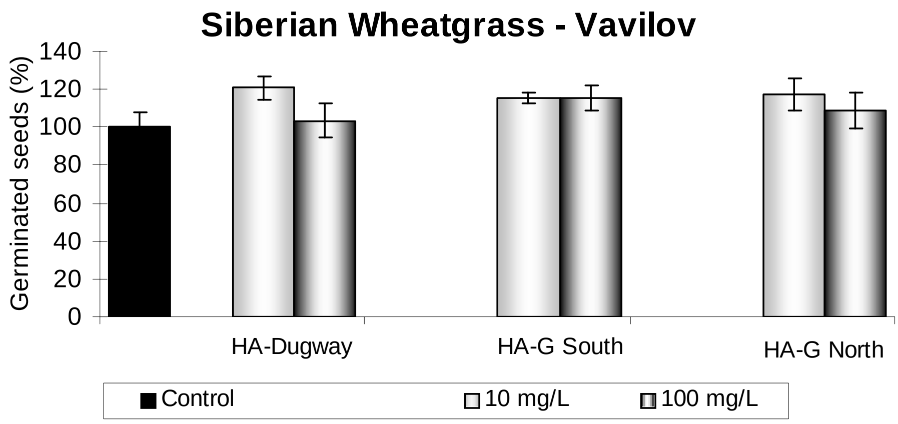


Figure 8. Fluorescence synchronous scan spectra of the humic acids isolated from the seven control greenhouse soils.



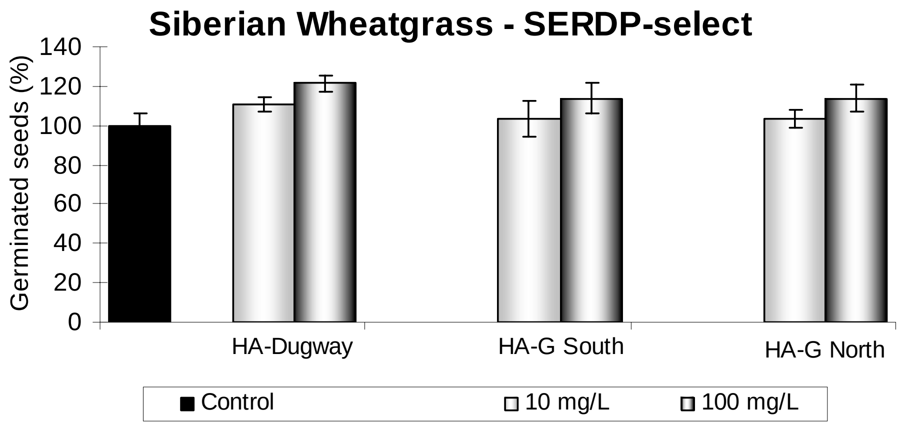
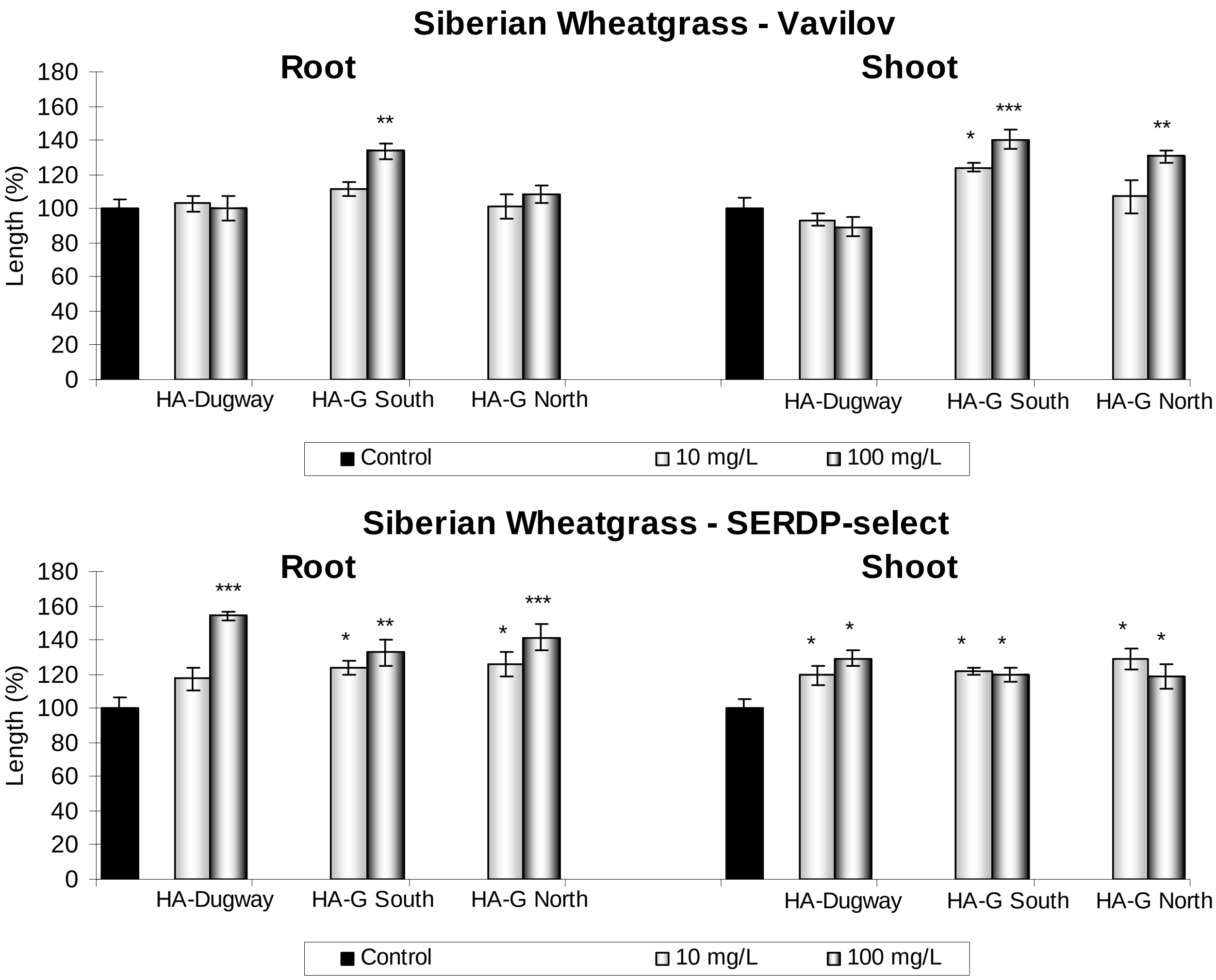


Figure 9. Effect of HAs at different concentrations on the number of germinated seeds expressed as percentages of control treatment (100 %). The vertical line on each bar indicates the standard error for 5 replicates.



*** $P \leq 0.001$; ** $P \leq 0.01$; * $P \leq 0.05$, according to the LSD test.

Figure 10. Effect of HAs at different concentrations on primary shoot and root length of germinated seeds, expressed as percentages of control treatment (100 %). The vertical line on each bar indicates the standard error for 5 replicates.

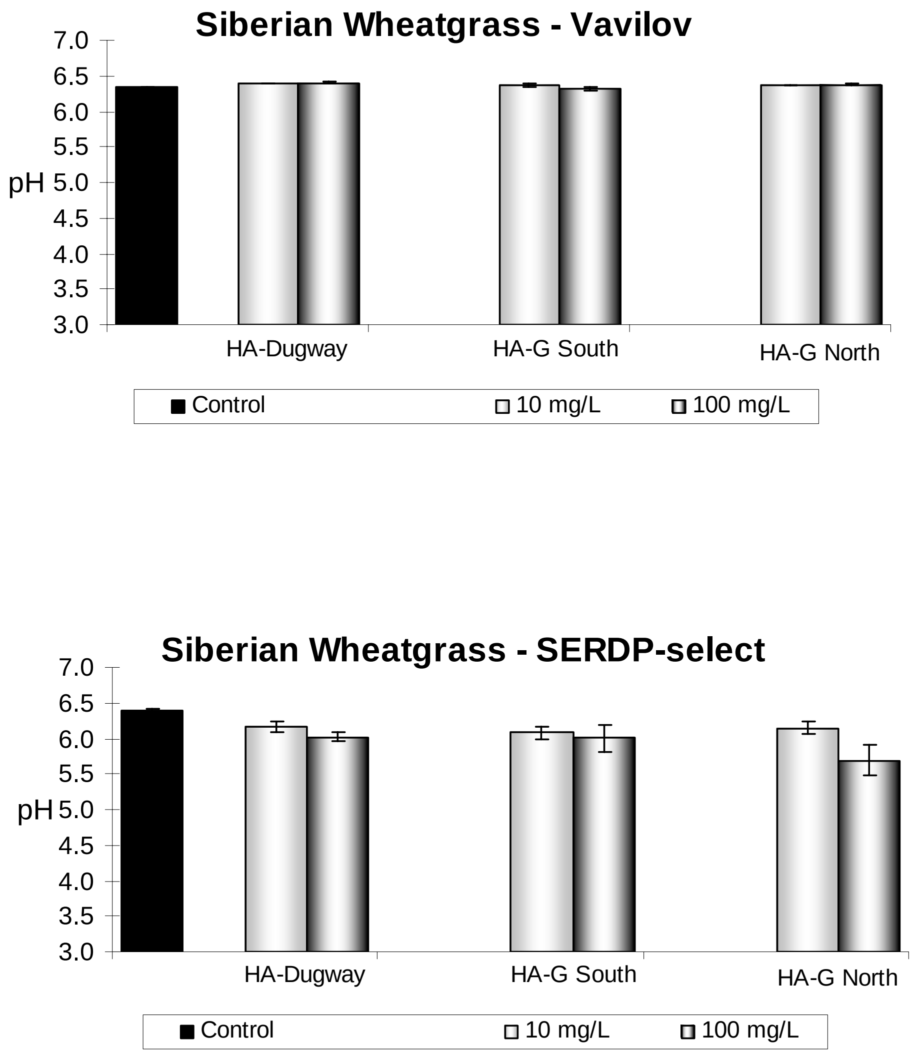
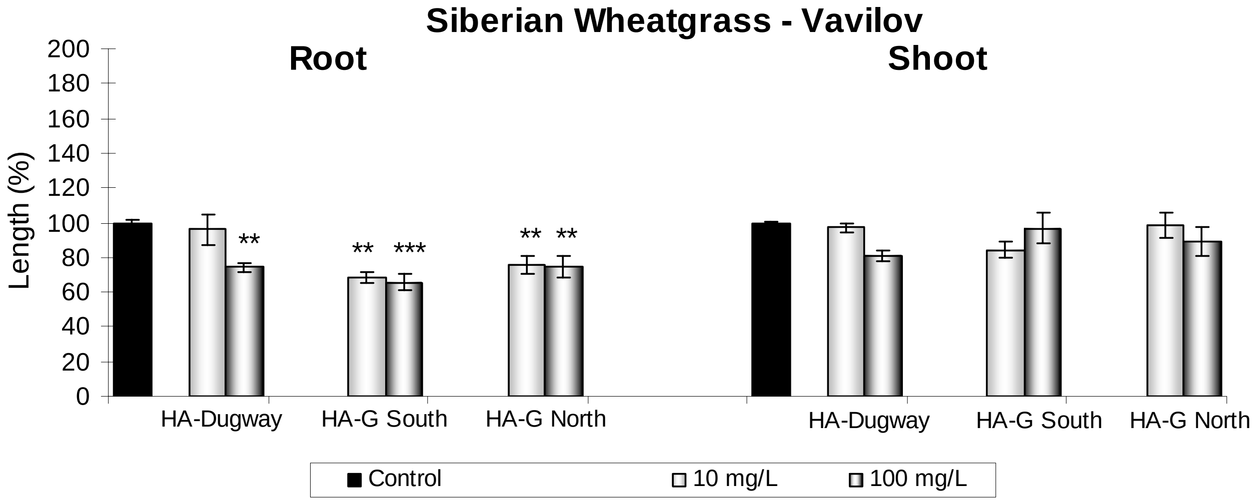
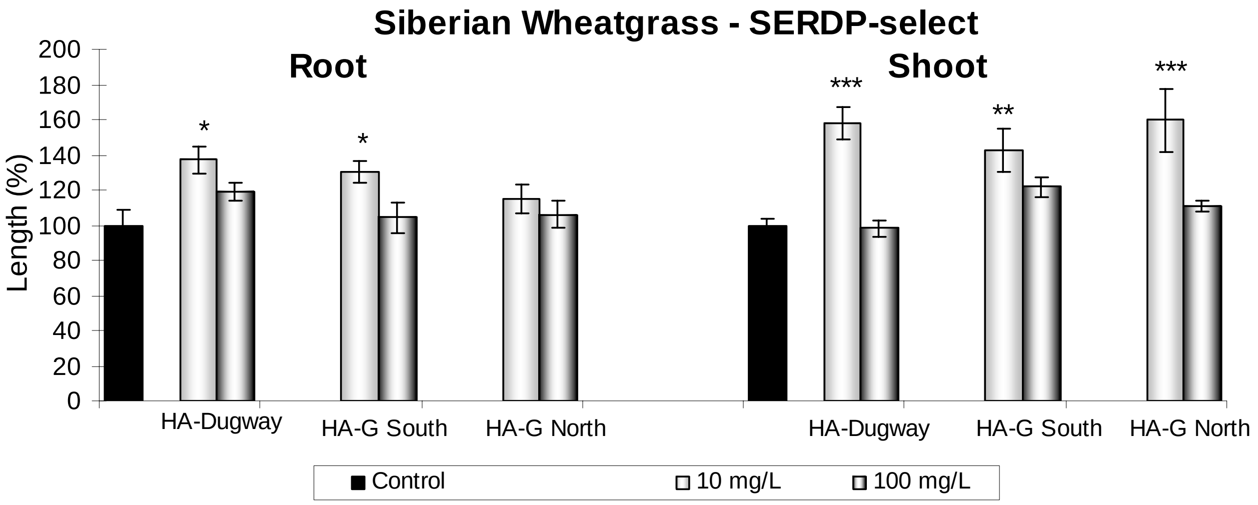


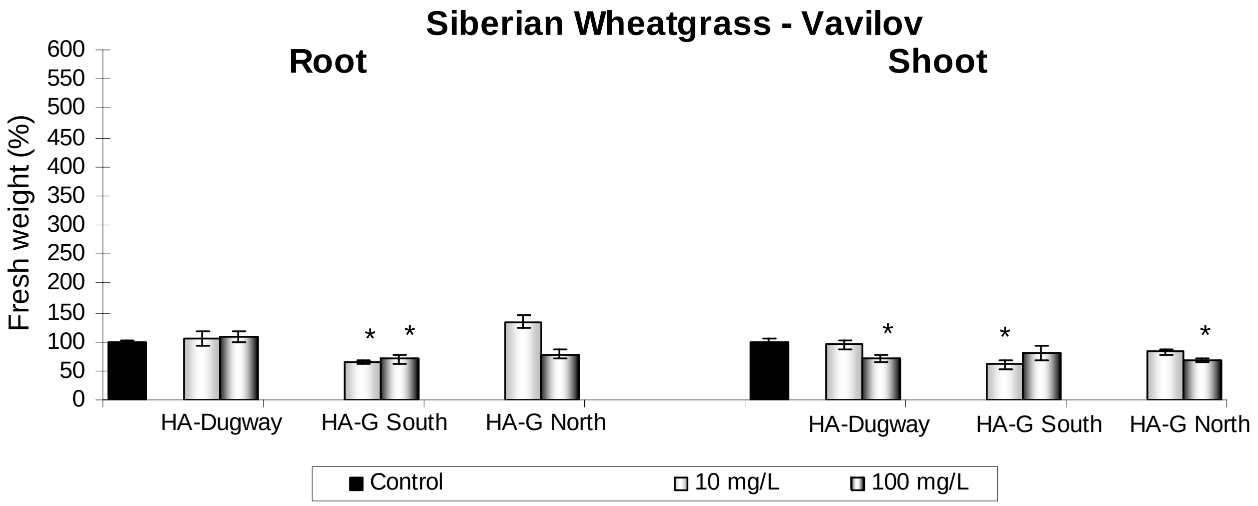
Figure 11. Effect of HAs at different concentrations on the pH value of growth medium measured after 21-day seedling growth. The vertical line on each bar indicates the standard error for 5 replicates.

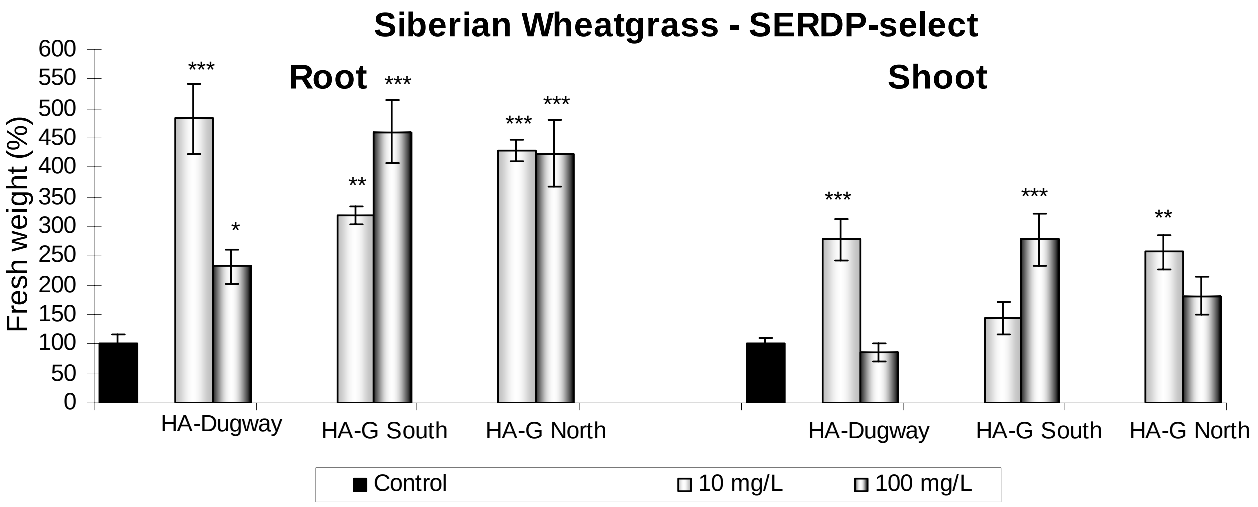




*** P ≤ 0.001; ** P ≤ 0.01; * P ≤ 0.05, according to the LSD test.

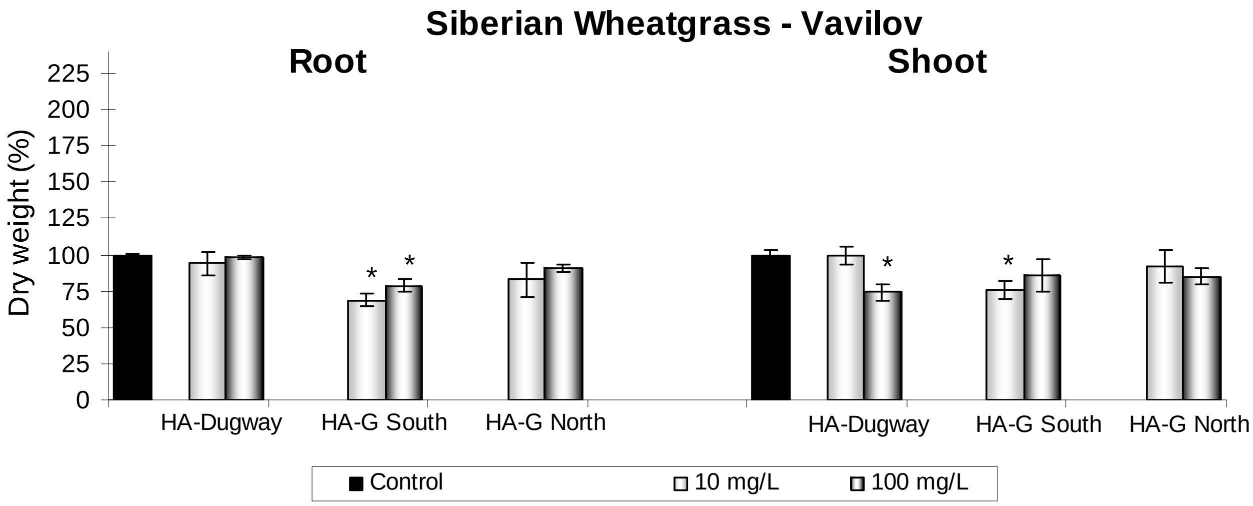
Figure 12. Effect of HAs at different concentrations on the length of shoots and roots expressed as percentages of control treatment (100 %) measured after 21-day seedling growth. The vertical line on each bar indicates the standard error for 5 replicates.

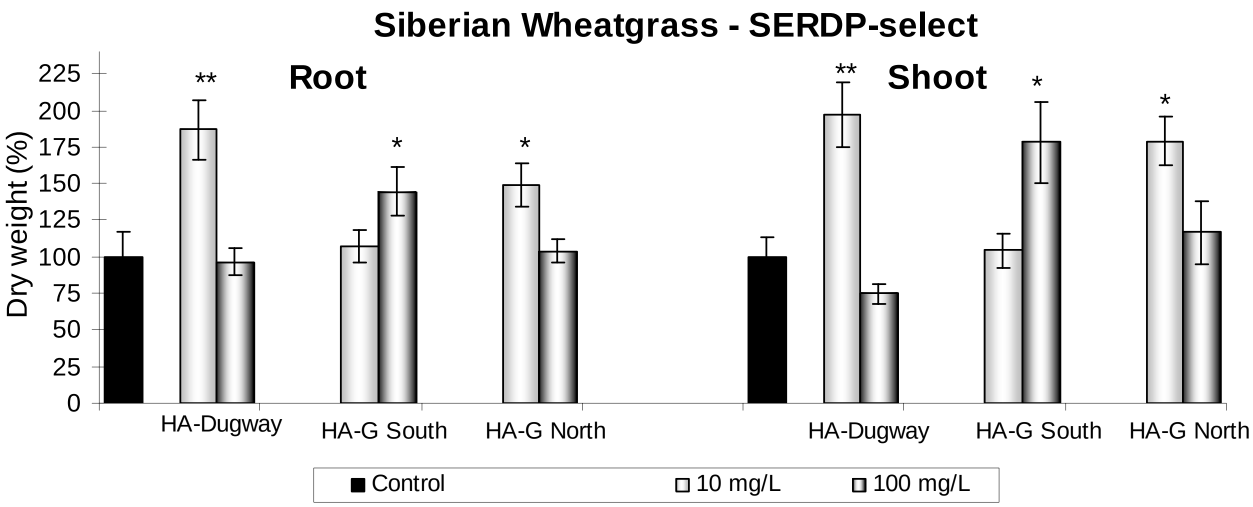




*** P ≤ 0.001; ** P ≤ 0.01; * P ≤ 0.05, according to the LSD test.

Figure 13. Effect of HAs at different concentrations on the fresh weight of shoots and roots expressed as percentages of control treatment (100 %) measured after 21-day seedling growth. The vertical line on each bar indicates the standard error for 5 replicates.





** P ≤ 0.01; * P ≤ 0.05, according to the LSD test.

Figure 14. Effect of HAs at different concentrations on the dry weight of shoots and roots expressed as percentages of control treatment (100 %) measured after 21-day seedling growth. The vertical line on each bar indicates the standard error for 5 replicates.